INFECTION AND IMMUNITY, Dec. 1999, p. 6341–6345 Vol. 67, No. 12
0019-9567/99/$04.00+0

Randomized, Double-Blind, Placebo-Controlled, Multicentered Trial of the Efficacy of a Single Dose of Live Oral Cholera Vaccine CVD 103-HgR in Preventing Cholera following Challenge with *Vibrio cholerae* O1 El Tor Inaba Three Months after Vaccination

CAROL O. TACKET,[1]* MITCHELL B. COHEN,[2] STEVEN S. WASSERMAN,[1] GENEVIEVE LOSONSKY,[1] SOFIE LIVIO,[1] KAREN KOTLOFF,[1] ROBERT EDELMAN,[1] JAMES B. KAPER,[1] STANLEY J. CRYZ,[3] RALPH A. GIANNELLA,[2] GILBERT SCHIFF,[2] AND MYRON M. LEVINE[1]

Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland 21201[1]; Children's Hospital Medical Center, University of Cincinnati, Cincinnati, Ohio 45229[2]; and Swiss Serum and Vaccine Institute, Berne, Switzerland[3]

Received 28 June 1999/Returned for modification 25 August 1999/Accepted 8 September 1999

CVD 103-HgR is a live oral cholera vaccine strain constructed by deleting 94% of the gene for the enzymatically active A subunit of cholera toxin from classical Inaba *Vibrio cholerae* O1 569B; the strain also contains a mercury resistance gene as an identifying marker. This vaccine was well tolerated and immunogenic in double-blind, controlled studies and was protective in open-label studies of volunteers challenged with *V. cholerae* O1. A randomized, double-blind, placebo-controlled, multicenter study of vaccine efficacy was designed to test longer-term protection of CVD 103-HgR against moderate and severe El Tor cholera in U.S. volunteers. A total of 85 volunteers (50 at the University of Maryland and 35 at Children's Hospital Medical Center/University of Cincinnati) were recruited for vaccination and challenge with wild-type *V. cholerae* El Tor Inaba. Volunteers were randomized in a double-blind manner to receive, with buffer, a single oral dose of either CVD 103-HgR (2×10^8 to 8×10^8 CFU) or placebo (killed *E. coli* K-12). About 3 months after immunization, 51 of these volunteers were orally challenged with 10^5 CFU of virulent *V. cholerae* O1 El Tor Inaba strain N16961, prepared from a standardized frozen inoculum. Ninety-one percent of the vaccinees had a ≥4-fold rise in serum vibriocidal antibodies after vaccination. After challenge, 9 (39%) of the 23 placebo recipients and 1 (4%) of the 28 vaccinees had moderate or severe diarrhea (≥3-liter diarrheal stool) ($P < 0.01$; protective efficacy, 91%). A total of 21 (91%) of 23 placebo recipients and 5 (18%) of 28 vaccinees had any diarrhea ($P < 0.001$; protective efficacy, 80%). Peak stool *V. cholerae* excretion among placebo recipients was 1.1×10^7 CFU/g and among vaccinees was 4.9×10^2 CFU/g ($P < 0.001$). This vaccine could therefore be a safe and effective tool to prevent cholera in travelers.

Cholera continues to be a major public health problem in nearly all developing countries, including countries in the Western hemisphere (2, 8, 26, 31). Cholera afflicts both children and adults and exists as an endemic disease in over 100 countries. Cholera does occur in the United States, but cases and deaths are rare. Travelers from the United States to areas where the disease is endemic are at some risk; this risk has been estimated to be about 1 per 30,000 travelers (37), although with active surveillance and use of optimal medium for isolation of *Vibrio cholerae*, the incidence of cholera in this population has been estimated to be much higher (36). For example, the incidence of cholera among U.S. citizens living in Lima, Peru, was 5.3 cases per 1,000 between 1991 and 1993 (36). The current parenteral vaccine, which provides about 50% protection, commonly elicits systemic and local adverse reactions (21). A more effective, better tolerated vaccine that could be administered orally is therefore desirable, and such new oral vaccines have been developed, including both killed vaccines and live attenuated strains (12, 13, 18, 19, 21). An ideal new vaccine would provide a high level of long-term protection to even those at high risk for severe illness, e.g., individuals with blood group type O. This protection would begin shortly after administration of a single oral dose (18, 19).

CVD 103-HgR is a vaccine strain constructed by deleting 94% of the gene encoding the enzymatically active A subunit of cholera toxin (*ctxA*) from classical Inaba *V. cholerae* 569B, leaving intact the gene encoding the immunogenic B (binding) subunit of cholera toxin (*ctxB*). A gene encoding resistance to Hg^{2+} ions was inserted into the El Tor hemolysin gene to provide a marker to differentiate the vaccine strain from wild-type *V. cholerae* classical Inaba strains, and an undefined, spontaneous mutation apparently arose during strain construction (14). This vaccine is well tolerated and immunogenic in diverse populations (5, 11, 15–17, 20, 25, 27, 32–34, 38); moreover, fecal excretion of this derivative is low, which should minimize environmental spread of the vaccine (20).

A single dose (5×10^8 CFU) of CVD 103-HgR has provided a high level of protection against cholera in groups of U.S. volunteers challenged with classical biotype *V. cholerae* O1 of either Inaba or Ogawa serotype (19, 20, 35). In the volunteer challenge model, significant efficacy against challenge with the homologous (classical) biotype persisted for at least 6 months after vaccination (the longest interval tested) and was present as soon as 8 to 10 days after vaccination (the shortest interval tested) (35). The current pandemic of cholera is due to *V. cholerae* of the El Tor biotype. In three open-label, unblinded efficacy studies of nonrandomized vaccinees ($n = 36$) and control volunteers ($n = 24$) challenged with El Tor *V. cholerae*, a

* Corresponding author. Mailing address: Center for Vaccine Development, University of Maryland School of Medicine, 685 West Baltimore St., Baltimore, MD 21201. Phone: (410) 706-5328. Fax: (410) 706-4171. E-mail: ctacket@medicine.umaryland.edu.

single dose of CVD 103-HgR provided complete protection against moderate and severe diarrhea (19, 20). Based on these volunteer studies in the United States, CVD 103-HgR was licensed in Canada and a number of European countries as a single-dose oral vaccine to prevent cholera.

We undertook a multicenter volunteer challenge study by using a randomized, placebo-controlled, double-blind method to determine the protective efficacy and confidence intervals for protection against moderate and severe diarrhea (≥3.0-liter purge) due to El Tor Inaba *V. cholerae* O1 at least 3 months after immunization with CVD 103-HgR.

MATERIALS AND METHODS

Study design. A total of 85 volunteers (50 at the University of Maryland and 35 at Children's Hospital Medical Center, University of Cincinnati) were enrolled and randomized to receive CVD 103-HgR or placebo, with the understanding that they would return for challenge with wild-type *V. cholerae* El Tor Inaba at least 3 months later. All volunteers received prestudy counseling and gave informed, written consent. To ensure comprehension of the study and to document that informed consent had been elicited, the volunteers had to pass a written examination before inoculation with the challenge strain. Volunteers were thoroughly screened to document their mental and physical health before challenge. The inclusion criteria for the challenge study were as follows: healthy man or woman, age 18 to 40 years; normal medical history and physical examination; and no clinically significant abnormalities of urinalysis, complete blood count, serum hepatic transaminases, glucose, creatinine, blood urea nitrogen, electrolytes, or electrocardiogram. The exclusion criteria were as follows: travel to a cholera endemic area in the previous 5 years; abnormal stool pattern or regular use of laxatives; failure to pass a psychological examination; allergy to tetracycline or ciprofloxacin; history of cholera or enterotoxigenic *E. coli* challenge; history of recent antibiotic use; pregnancy or nursing; positive serology for HIV, hepatitis B antigen, or hepatitis B; stool culture positive for an enteric pathogen; or failure to pass the written examination.

Method of randomization. For those of blood group O and non-O within each clinical center, subjects were randomized in blocks of four (two to receive vaccine and two to receive placebo) by using SAS PROC PLAN. Volunteers were stratified by blood group (O versus non-O), because persons of blood group O are predisposed to develop more severe forms of cholera diarrhea (3, 9). The code was held by the study sponsor until the database was complete and unalterable.

Vaccination. Volunteers were randomized in a double-blind manner to receive, with buffer, a single oral dose of either CVD 103-HgR (2×10^8 to 8×10^8 CFU) ($n = 43$) or placebo (killed *E. coli* K-12) ($n = 42$). For 3 days after vaccination as outpatients, the volunteers kept a symptom diary to record all stools and to determine the occurrence of adverse reactions such as diarrhea, nausea, vomiting, abdominal cramps, malaise, anorexia, headache, and fever.

Vaccine and placebo formulations. The vaccine strain was prepared and packaged by the Swiss Serum and Vaccine Institute as a lyophilized preparation. The formulation consisted of a pair of attached sachets, one containing a single dose (2×10^8 to 8×10^8 viable organisms) of lyophilized vaccine and the other containing effervescent buffer powder to be mixed with vaccine in 100 ml of water. Each sachet of buffer contained $NaHCO_3$ (2.5 g), ascorbic acid (1.65 gm), and aspartame (25 mg). The placebo formulation consisted of an identical-appearing pair of sachets, one containing killed *E. coli* K-12 and the other containing buffer. When suspended in the buffer solution, the placebo was identical in appearance to the vaccine suspension.

The sachet containing buffer and the sachet containing test product (either vaccine or placebo) were emptied into a cup containing 100 ml of distilled water and mixed. Volunteers had nothing by mouth for 30 min before and after ingestion of the vaccine or placebo.

Challenge. Approximately 3 months after immunization (mean, 113 days; median, 109 days; range, 85 to 143 days), 51 volunteers (28 vaccinees and 23 placebo recipients) were admitted to Kernan Hospital in Baltimore or to the General Clinical Research Center at Children's Hospital Medical Center in Cincinnati to be orally challenged with 10^5 CFU of virulent *V. cholerae* O1 El Tor Inaba strain N16961, prepared from a standardized frozen inoculum (30). During a period of 1 to 2 days before ingestion of the challenge inoculum, the volunteers were acclimated to the ward while medical screening was completed. Baseline samples of serum were collected for the measurement of antibody.

The El Tor Inaba *V. cholerae* O1 challenge strain N16961 was thawed and diluted to 10^5 organisms per ml. The inoculum size was quantitated by the replica spread-plate technique before and after challenge. Two grams of $NaHCO_3$ was dissolved in 150 ml of distilled water. Volunteers drank 120 ml of the $NaHCO_3$ water; 1 min later, they ingested 10^5 CFU of the challenge strain suspended in the remaining 30 ml of $NaHCO_3$ water. Volunteers had nothing by mouth for 90 min before and after challenge.

For a period of 96 h after the ingestion of vibrios, the volunteers were closely monitored to detect any adverse reactions. An investigator interviewed the volunteers at least once daily. Every stool was saved, examined, graded, and if loose, weighed. The consistency of the stool was graded according to five grades: grade 1, firm; grade 2, soft; grade 3, thick liquid; grade 4, opaque watery; and grade 5, rice water. The total diarrheal stool volume (stool grades 3 to 5) was determined, including loose stools that occurred after tetracycline therapy had begun. The first 10 stools passed each day were cultured to detect the excretion of the challenge strain.

Any volunteer who developed diarrhea after challenge received oral glucose/electrolytes solution to prevent dehydration. Oral rehydration was given in a volume 1.5 times the diarrheal stool volume after each loose stool. Intravenous rehydration with a balanced polyelectrolyte solution was administered to three volunteers with diarrhea. Tetracycline, 500 mg four times a day for 5 days, was given when the volunteers exceeded 5.0 liters of total diarrheal stool output or on day 4, whichever occurred first. Volunteers were discharged when they were asymptomatic, when they had received a course of tetracycline, and when their stool cultures were negative for *V. cholerae* for 3 consecutive days beginning 72 h after inoculation.

Definitions of illness. For outpatient volunteers after administration of vaccine or placebo, diarrhea was defined as four loose stools in a 24-h period. For subjects under inpatient surveillance after challenge, diarrhea was defined as the passage of two or more unformed (grades 3 to 5) stools over a 48-h period that equaled or exceeded 200 ml or a single stool of 300 ml or greater.

A case of cholera was defined as a subject with a positive stool culture for *V. cholerae* O1 who met the above-mentioned definition of diarrhea. A moderate case of cholera was one who passed at least 3 liters of diarrheal stool (grades 3 to 5) during the study, and a severe case was one who passed at least 5 liters during the study. Fever was defined as an oral temperature of >38°C.

Serology. Blood was collected before and 10 days after vaccination to provide sera for measurement of vibriocidal antibodies and antibodies to cholera toxin. Blood was collected before and on days 9 and 14 after challenge for serologic studies. Coded sera from each subject were tested for vibriocidal antibodies by using *V. cholerae* O1 El Tor Inaba strain 89 and for immunoglobulin G (IgG) and IgA antitoxin by enzyme-linked immunosorbent assay (ELISA) (1, 4, 23, 24, 29). A fourfold or greater rise in serum vibriocidal titer over that of the baseline specimen was considered significant. For anti-CT antibody, a ≥0.20 rise in optical density units between pre- and postvaccination or challenge specimens was considered significant.

Bacteriology. All stools after challenge were plated directly onto thiosulfate citrate bile salts sucrose (TCBS) agar, as well as inoculated into alkaline peptone water enrichment broth, for overnight incubation before plating onto TCBS agar (28). Up to two stools each day were cultured quantitatively to determine the number of vaccine organisms per gram of stool. A rectal swab was obtained if no stool was passed. Suspicious colonies were agglutinated with specific *V. cholerae* O1 Inaba antiserum.

Statistical analysis. The frequency of adverse reactions among vaccinees and placebo recipients was compared by use of the Fisher's exact test (FET). The maximum recorded severity for each of these reactions was compared between groups by use of the Wilcoxon test. The total number of days of diarrhea and peak temperature among vaccinees and placebo recipients were compared by using Wilcoxon tests.

The vibriocidal and anti-cholera toxin seroconversion rates were compared between vaccinees and placebo recipients by FET. Pre- and postimmunization vibriocidal antibody titers were compared between groups by using Wilcoxon tests.

The point estimate of protective efficacy was calculated as the difference in attack rates among placebo recipients and vaccinees divided by the attack rate among placebo recipients. Confidence limits on protective efficacy were calculated by using methods based on the likelihood score statistic (7).

The study was designed to have 80% power to detect an 80% protective efficacy against moderate and severe diarrhea by using a two-tailed analysis evaluated at $P = 0.05$.

TABLE 1. Incidence of symptoms within 3 days of receiving CVD 103-HgR live oral cholera vaccine or placebo

Symptom	No. of subjects with symptom/total no. (%)		
	Vaccinees ($n = 43$)	Placebo recipients ($n = 42$)	P
Anorexia	14/43 (33)	7/42 (17)	0.13
Malaise	10/43 (23)	14/42 (33)	0.34
Abdominal cramps	12/43 (28)	12/42 (29)	1.00
Headache	15/43 (35)	13/42 (31)	0.82
Vomiting	5/43 (12)	1/42 (2)	0.20
Nausea	10/43 (23)	9/42 (21)	1.00
Diarrhea	2/43 (5)	1/42 (2)	1.00
Fever	0/42 (0)	0/42 (0)	

TABLE 2. Immune response to vaccination with live oral cholera vaccine CVD 103-HgR as measured by serum Inaba vibriocidal reciprocal GMT and by the mean optical density for anti-cholera toxin assays

Group	Inaba vibriocidal reciprocal titer (range)			Anti-cholera toxin IgG (range)		
	Seroconversion rate (%)	Pre	Post	Seroconversion rate (%)	Pre	Post
All vaccinees ($n = 43$)	91	27 (10–640)	3,056 (20–20,480)	51	0.12 (0.01–0.83)	0.67 (0.03–2.43)
All placebo recipients ($n = 42$)	2	23 (10–640)	26 (10–640)	5	0.14 (0.00–0.38)	0.15 (0.02–0.41)

RESULTS

Volunteers. The 43 vaccinees and 42 placebo recipients had similar demographic profiles. The participants included 48 men and 37 women with an average age of 30.1 years (range, 18 to 40 years). A total of 40% of the vaccinees and 41% of the placebo recipients were of blood group O. Three months after vaccination, 56 of the 85 volunteers were available, willing, and medically and psychologically eligible to participate in the cholera challenge study. Of these, 51 were randomized to participate in the challenge phase; 33 were challenged at the University of Maryland, and 18 were challenged at the University of Cincinnati. Among the 51 subjects, 54% of the 28 vaccinees and 35% of the 23 placebo recipients were of blood group O (P was not significant). There were no statistically significant demographic differences between subjects who participated in the challenge study and those who did not.

Reactions to vaccine. The vaccine was well tolerated. There were no significant differences in the rates or severity of anorexia, malaise, abdominal cramps, headache, nausea, vomiting, diarrhea, or fever reported by subjects who had ingested CVD 103-HgR and those who had ingested killed *E. coli* K-12 placebo (Table 1).

Immune response to vaccine. Overall, 91% of the vaccine recipients developed significant increases in serum Inaba vibriocidal antibody titers. The geometric mean reciprocal titer (GMT) was 3,056 (range, 20 to 20,480) for all vaccinees after vaccination, a 113-fold rise over the baseline GMT (Table 2). Among vaccinees who participated in the challenge phase, 93% had significant rises in Inaba vibriocidal titers with a GMT of 1,470 (range, 20 to 20,480) after vaccination. One volunteer experienced a rise in vibriocidal titer after receiving placebo; he remained clinically well during this period. This response may represent cross-reacting antibodies to some subclinical infection (6) or, less likely, a specimen collection, laboratory, or protocol error. After immunization, serum IgG anti-cholera toxin rose in 61% of vaccinees who subsequently underwent challenge.

Clinical and bacteriologic responses to challenge. A total of 9 (39%) of the 23 placebo recipients and 1 (4%) of the 28 vaccinees developed moderate or severe cholera (≥3.0-liters of total diarrheal stool volume) after challenge ($P = 0.003$; protective efficacy, 91%; lower 95% confidence limit, 51%) (Table 3). The one vaccinee who became ill had severe cholera with a total diarrheal stool volume of 6.8 liters. She was of blood group O and had had a vigorous vibriocidal antibody response to vaccine (titer of 1:20,480 on day 10 after vaccination) and, immediately before challenge 3 months later, she had a vibriocidal titer of 1:1,280.

Twenty-one (91%) of 23 placebo recipients and 5 (18%) of the 28 vaccinees had diarrhea of any volume ($P < 0.001$; protective efficacy, 80%; lower 95% confidence limit, 60%). The mean diarrheal stool volumes and the numbers of diarrheal stools did not differ statistically between vaccinees and placebo recipients, but placebo recipients were more likely to have fever (Table 3). Peak stool *V. cholerae* O1 excretion among placebo recipients was 1.1×10^7 CFU/g and among vaccinees was 4.9×10^2 CFU/g ($P < 0.001$) (Table 3).

Relationship between immune responses to vaccination and protection against cholera challenge. Among the 28 vaccinees, the vibriocidal antibody titers measured 10 days after immunization and those measured immediately before challenge were examined in relation to whether or not the vaccinee had diarrhea and to the severity of diarrhea after challenge. Neither the 10-day postvaccination vibriocidal titer nor the vibriocidal titer drawn immediately before challenge clearly correlated with incidence or severity of illness after challenge among the vaccinees (Table 4).

TABLE 3. Clinical and bacteriologic responses to challenge with *V. cholerae* O1 El Tor Inaba strain N16961 after receipt of CVD 103-HgR vaccine or placebo

Parameter	Vaccinees			Placebo recipients			P
	Total	Blood group O	Blood group non-O	Total	Blood group O	Blood group non-O	
n (%)	28	15 (54)	13 (46)	23	8 (35)	15 (65)	0.26[a]
Moderate or severe cholera (≥3-liter diarrheal stool) (%)	1 (4)	1 (7)	0 (0)	9 (39)	4 (50)	5 (33)	0.003[b]
Any diarrhea (%)	5 (18)	4 (27)	1 (8)	21 (91)	7 (88)	14 (93)	<0.001[b]
Mean diarrheal stool volume in liters for ill volunteers (range)	2.0 (0.7–6.8)	2.3 (0.7–6.8)	0.7	3.5 (0.3–8.2)	4.1 (0.5–7.1)	3.2 (0.3–8.2)	0.11[b]
No. of diarrheal stools for ill volunteers (range)	13 (6–42)	15 (6–42)	6	23 (2–63)	28 (4–63)	20 (2–55)	0.14[b]
Incidence of fever (%)	0	0	0	6 (35)	0	6	0.006[b]
Stool *V. cholerae* O1 excretion (%)	17 (61)	11 (73)	6 (46)	22 (96)	7 (88)	6 (40)	0.003[b]
Mean peak stool *V. cholerae* O1 excretion for all volunteers (CFU/g)	4.9×10^2	1.7×10^3	1.2×10^2	1.1×10^7	6.0×10^6	1.4×10^7	<0.001[b]

[a] P value comparing proportion of volunteers of blood group O among vaccinees and placebo recipients.
[b] P value comparing all vaccinees and all placebo recipients.

TABLE 4. Relationship between geometric mean reciprocal Inaba vibriocidal titer on day 10 after immunization and titer immediately before challenge and protection against experimental challenge with *V. cholerae* O1 El Tor Inaba among all vaccinees, vaccinees of blood group O, and vaccinees of non-O blood groups

Symptom group	All vaccinees (n = 28)			Vaccinees of blood group O (n = 15)			Vaccinees of non-O blood groups (n = 13)		
	n	Titer 10 days after immunization	Titer before challenge	n	Titer 10 days after immunization	Titer before challenge	n	Titer 10 days after immunization	Titer before challenge
No diarrhea	23	4,968	244	11	6,185	341	12	4,064	180
Mild diarrhea	4	1,810	95	3	1,280	101	1	5,120	80
Moderate or severe diarrhea	1	20,480	1,280	1	20,480	1,280	0		

DISCUSSION

Multiple previous studies of the efficacy of CVD 103-HgR against challenge with El Tor and classical *V. cholerae* O1 have been conducted among U.S. volunteers (19, 20, 35). These studies were open-label, unblinded, nonrandomized studies in which vaccinees were enrolled and vaccinated 1 to 6 months before challenge and, for logistical reasons, unvaccinated control volunteers were enrolled at a later time just before challenge. In these studies, the prospectively designated outcome measure was the development of moderate or severe cholera, which was defined by specific volumes of diarrheal stool; moderate cholera was defined as 3 liters of diarrheal stool, and severe cholera was defined as 5 liters of diarrheal stool. The entire blood volume of an average adult male is ca. 5 liters, so these volumes represent potentially life-threatening fluid losses if such diarrhea were untreated. Because of the objective outcome measure used in this study design, it was considered unlikely that the outcome was significantly affected by investigator or volunteer bias.

However, the rigorous randomized double-blind design of the current study allays concerns about any possible biases that might have been inadvertently introduced in the previous studies. Moreover, the results of this trial increase the experience in protecting against El Tor cholera, the biotype which is currently prevalent throughout the world. In this rigorous examination of vaccine efficacy, CVD 103-HgR performed well; the vaccine induced 91% protective efficacy against moderate and severe El Tor cholera, the clinical syndrome that is responsible for mortality associated with the disease, and provided 80% efficacy against any diarrhea after El Tor *V. cholerae* O1 challenge in these U.S. volunteers, a level higher than that observed in previous studies (19). Previous studies have shown no difference in protection after rechallenge with *V. cholerae* of different serotypes (Ogawa or Inaba) (22). In addition, this trial demonstrates that the duration of protection against El Tor cholera provided by CVD 103-HgR extends to at least 3 months. The vaccine, as expected, reduced the extent of excretion of vibrios in the stools of challenged volunteers by 100,000-fold.

For the first time in an efficacy study of CVD 103-HgR, a vaccinated volunteer developed severe cholera after challenge. This was not due to a failure of the subject to respond serologically to the vaccine. This volunteer, a healthy 32-year-old woman of blood group O, had vigorous anti-cholera toxin and Inaba vibriocidal antibody responses 10 days after immunization, in which her vibriocidal titer rose from 1:10 to 1:20,480. The vibriocidal titer immediately before challenge 3 months later was 1:1,280. Serum vibriocidal titer has been considered a surrogate marker of protective immunity, and a titer of this level would have been expected to protect her from at least severe diarrhea (10). The absence of protection shows that the vibriocidal response is an imperfect surrogate measure of protection at the mucosa in any individual patient. Retrospectively, total serum and stool IgA levels were measured in this volunteer and were within the normal range, so that IgA deficiency did not contribute to the severe clinical syndrome she experienced after challenge.

In reviewing the entire experience over several years, only 1 (1%) of 103 CVD 103-HgR vaccinees developed moderate or severe cholera after challenge with classical or El Tor biotype *V. cholerae* compared to 21 (24%) of 86 control volunteers. When challenges with El Tor strains only are reviewed in combination, only 1 (2%) of 64 vaccinees but 21 (45%) of 47 control volunteers experienced moderate or severe cholera. Although strict statistical analysis of these data is problematic since the studies were designed differently and conducted over a period of many years, the overwhelming impression is supportive of the strong protective effect of vaccination.

The collective experience of experimental cholera in U.S. volunteers vaccinated with CVD 103-HgR is in direct contrast to the only field trial of the efficacy of CVD 103-HgR carried out in an area where the disease is endemic. In Jakarta, Indonesia, protection could not be detected for 6 to 53 months after vaccination, and too few cases occurred in the first 6 months to determine whether or not protection occurred during this period (29). Experimental cholera challenge is arguably a better model for estimating efficacy among immunologically naive travelers from developed countries to countries where the disease is endemic than a direct measurement of field efficacy among residents of such an area. Therefore, we think the weight of evidence gathered through challenge studies suggests that CVD 103-HgR may be a suitable vaccine for protecting travelers against cholera.

ACKNOWLEDGMENTS

This study was supported by contracts NO1 AI-45251 and NO1-AI-45252 from the National Institute of Allergy and Infectious Diseases (NIAID), MO1-RR08084 from the NCRR-NIH, and the Swiss Serum and Vaccine Institute.

We gratefully acknowledge the generous contributions of our community volunteers from Baltimore and Cincinnati. We are also grateful for the contributions of Maria DeLoria of the Clinical Regulatory Affairs Branch of DMID, NIAID; Susan Parker and Kathy Palmer, who coordinated this study at the two sites; and the nursing staffs of the General Clinical Research Center, Children's Hospital Medical Center, and the Center for Vaccine Development for their expertise in clinical investigation.

REFERENCES

1. **Benenson, A. S., A. Saad, and W. H. Mosley.** 1968. Serological studies in cholera. The vibriocidal response in cholera patients determined by a microtiter technique. Bull. W.H.O. **38:**277–285.
2. **Blake, P. A.** 1993. Epidemiology of cholera in the Americas. Gastroenterol. Clin. North Am. **3:**639–660.
3. **Clemens, J. D., D. A. Sack, J. R. Harris, J. Chakraborty, M. R. Khan, S. Huda, F. Ahmed, J. Gomes, M. R. Rao, and A. M. Svennerholm.** 1989. ABO blood groups and cholera: new observations on specificity of risk and mod-

ifications of vaccine efficacy. J. Infect. Dis. **159:**770–773.
4. **Clements, M. L., M. M. Levine, C. R. Young, R. E. Black, Y.-L. Lim, and R. M. Robins-Browne.** 1982. Magnitude, kinetics and duration of vibriocidal antibody response in North Americans after ingestion of *Vibrio cholerae*. J. Infect. Dis. **145:**465–473.
5. **Cryz, S. J., Jr., M. M. Levine, J. B. Kaper, E. Fürer, and B. Althaus.** 1990. Randomized, double-blind, placebo-controlled trial to evaluate the safety and immunogenicity of the live oral cholera vaccine strain CVD 103-HgR in Swiss adults. Vaccine **8:**577–580.
6. **Gangarosa, E. J., W. E. DeWitt, J. C. Feeley, and M. R. Adams.** 1970. Significance of vibriocidal antibodies with regard to immunity to cholera. J. Infect. Dis. **121:**S36–S43.
7. **Gart, J. J., and J. Nam.** 1988. Approximate interval estimation of the ratio of binomial parameters: a review and corrections for skewness. Biometrics **44:**323–338.
8. **Glass, R. I., M. Claeson, P. A. Blake, R. J. Waldman, and N. F. Pierce.** 1991. Cholera in Africa: lessons on transmission and control for Latin America. Lancet **338:**791–795.
9. **Glass, R. I., J. Holmgren, C. E. Haley, M. R. Khan, A. M. Svennerholm, B. J. Stoll, K. M. Belayet Hossain, R. E. Black, M. Yunus, and D. Barua.** 1985. Predisposition for cholera of individuals with O blood group. Possible evolutionary significance. Am. J. Epidemiol. **121:**791–796.
10. **Glass, R. I., A. M. Svennerholm, M. R. Khan, S. Huda, M. I. Huq, and J. Holmgren.** 1985. Seroepidemiological studies of El Tor cholera in Bangladesh: association of serum antibody levels with protection. J. Infect. Dis. **151:**236–242.
11. **Gotuzzo, E., B. Butron, C. Seas, M. Penny, R. Ruiz, G. Losonsky, D. Lanata, S. Wasserman, E. Salazar, J. Kaper, S. Cryz, and M. Levine.** 1993. The safety, immunogenicity and excretion pattern of single-dose live oral cholera vaccine, CVD 103-HgR in Peruvian adults of high and low socioeconomic level. Infect. Immun. **61:**3994–3997.
12. **Holmgren, J., A. M. Svennerholm, M. Jertborn, J. Clemens, D. A. Sack, R. Salenstedt, and H. Wigzell.** 1992. An oral B subunit:whole cell vaccine against cholera. Vaccine **10:**911–914.
13. **Kenner, J. R., T. A. Coster, D. N. Taylor, A. F. Trofa, M. Barrera-Oro, T. Hyman, J. M. Adams, D. T. Beattie, K. P. Killeen, and D. R. Spriggs.** 1995. Peru-15, an improved live attenuated oral vaccine candidate for *Vibrio cholerae* O1. J. Infect. Dis. **172:**1126–1129.
14. **Ketley, J. M. J. Michalski, J. Galen, M. M. Levine, and J. B. Kaper.** 1993. Construction of genetically marked *Vibrio cholerae* O1 vaccine strains. FEMS Microbiol. Lett. **111:**15–22.
15. **Kotloff, K. L., S. S. Wasserman, S. A. O'Donnell, G. A. Losonsky, S. J. Cryz, and M. M. Levine.** 1992. Safety and immunogenicity in North Americans of a single dose of live oral cholera vaccine CVD 103-HgR: results of a randomized, placebo-controlled, double-blind cross-over trial. Infect. Immun. **60:**4430–4432.
16. **Lagos, R., A. Avendaño, I. Horwitz, V. Prado, C. Ferreccio, V. Sotomayor, G. Losonsky, S. Wasserman, S. Cryz, J. B. Kaper, and M. Levine.** 1993. Tolerance and immunogenicity of an oral dose of CVD 103-HgR, a live attenuated *Vibrio cholerae* O1 strain: a double-blind study in Chilean adults. Rev. Med. Chile **121:**857–863.
17. **Lagos, R., A. Avendaño, V. Prado, I. Horwitz, S. S. Wasserman, G. Losonsky, S. J. Cryz, Jr., J. B. Kaper, and M. M. Levine.** 1995. Attenuated live oral cholera vaccine strain CVD 103-HgR elicits significantly higher serum vibriocidal antibody titers in persons of blood group O. Infect. Immun. **63:**707–709.
18. **Levine, M. M., and J. B. Kaper.** 1993. Live oral vaccines against cholera: an update. Vaccine **11:**207–212.
19. **Levine, M. M., and J. B. Kaper.** 1995. Live oral cholera vaccine: from principle to product. Bull. Inst. Pasteur **93:**243–353.
20. **Levine, M. M., J. B. Kaper, D. Herrington, J. Ketley, G. Losonsky, C. O. Tacket, B. Tall, and S. Cryz.** 1988. Safety, immunogenicity, and efficacy of recombinant live oral cholera vaccines, CVD 103 and CVD 103-HgR. Lancet **ii:**467–470.
21. **Levine, M. M., and N. F. Pierce.** 1992. Immunity and vaccine development, p. 285–327. *In* W. B. Greenough III and D. Barua (ed.), Cholera. Plenum Press, New York, N.Y.
22. **Levine, M. M., and C. O. Tacket.** 1995. Live oral vaccines against cholera, p. 233–258. *In* D. A. A. Ala'Aldeen and C. E. Hormaeche (ed.), Molecular and clinical aspects of bacterial vaccine development. John Wiley & Sons, Chichester, England.
23. **Levine, M. M., C. R. Young, R. E. Black, Y. Takeda, and R. A. Finkelstein.** 1985. Enzyme-linked immunosorbent assay to measure antibodies to purified heat-labile enterotoxins from human and porcine strains and to cholera toxin: application in serodiagnosis and seroepidemiology. J. Clin. Microbiol. **21:**174–179.
24. **Levine, M. M., C. R. Young, T. P. Hughes, S. O'Donnell, R. E. Black, M. L. Clements, R. Robins-Browne, and Y.-L. Lim.** 1981. Duration of serum antitoxin response following *Vibrio cholerae* infection in North Americans: relevance for seroepidemiology. Am. J. Epidemiol. **114:**348–354.
25. **Migasena, S., P. Pitisuttitham, B. Prayurahong, P. Suntharasami, W. Supanaranond, V. Desakorn, U. Vongsthongsri, B. Tall, J. Ketley, G. Losonsky, S. Cryz, J. B. Kaper, and M. M. Levine.** 1989. Preliminary assessment of the safety and immunogenicity of live oral cholera vaccine strain CVD 103-HgR in healthy Thai adults. Infect. Immun. **57:**3261–3264.
26. **Mintz, E. D., R. V. Tauxe, and M. M. Levine.** 1998. The global resurgence of cholera, p. 63–104. *In* N. Noah and M. O'Mahony (ed.), Communicable disease: epidemiology and control. John Wiley & Sons, Chichester, England.
27. **Perry, R. T., C. V. Plowe, B. Koumaré, K. L. Kotloff, G. A. Losonsky, S. S. Wasserman, and M. M. Levine.** 1998. A single dose of live oral cholera vaccine CVD 103-HgR is safe and immunogenic in HIV-infected and non-infected adults in Mali. Bull. W.H.O. **76:**63–71.
28. **Rennels, M. B., M. M. Levine, V. Daya, P. Angle, and C. Young.** 1980. Selective vs. non-selective media and direct plating vs. enrichment technique in isolation of *Vibrio cholerae*: recommendations for clinical laboratories. J. Infect. Dis. **142:**328–331.
29. **Richie, E., N. H. Punjabi, Y. Sidharta, K. Peetosutan, M. Sukandar, S. S. Wasserman, M. Lesmana, Wangsasaputra, S. Pandam, M. M. Levine, P. O'Hanley, S. J. Cryz, and C. H. Simanjuntak.** Efficacy trial of single-dose live oral cholera vaccine CVD 103-HgR in North Jakarta, Indonesia, a cholera-endemic area. Submitted for publication.
30. **Sack, D. A., C. O. Tacket, M. B. Cohen, R. B. Sack, G. A. Losonsky, J. Shimko, J. P. Nataro, R. Edelman, M. M. Levine, R. A. Giannella, G. Schiff, and D. Lang.** 1998. Validation of a volunteer model of cholera with frozen bacteria as the challenge. Infect. Immun. **66:**1968–1972.
31. **Sanchez, J. L., and D. N. Taylor.** 1997. Cholera. Lancet **349:**1825–1830.
32. **Simanjuntak, C. H., P. O'Hanley, N. H. Punjabi, F. Noriega, G. Pazzaglia, P. Dykstra, B. Kay, C. Suharyono, A. Budiarso, A. Rifai, S. S. Wasserman, G. A. Losonsky, J. Kaper, S. Cryz, and M. M. Levine.** 1993. The safety, immunogenicity and transmissibility of single-dose live oral cholera vaccine CVD 103-HgR in 24 to 59 month old Indonesian children. J. Infect. Dis. **168:**1169–1176.
33. **Su-Arehawaratana, P. S., P. Singharaj, D. N. Taylor, C. Hoge, A. Trofa, K. Kuvanont, S. Migasena, P. Pitisuttitham, Y.-L. Lim, G. Losonsky, J. B. Kaper, S. S. Wasserman, S. Cryz, P. Echeverria, and M. M. Levine.** 1992. Safety and immunogenicity of different immunization regimens of CVD 103-HgR live oral cholera vaccine in soldiers and civilians in Thailand. J. Infect. Dis. **165:**1042–1048.
34. **Suharyono, C., C. H. Simanjuntak, N. Witham, N. Punjabi, D. G. Heppner, G. A. Losonsky, H. Totosudirjo, A. R. Rifai, J. Clemens, Y. L. Lim, D. Burr, S. S. Wasserman, J. Kaper, K. Sorenson, S. Cryz, and M. M. Levine.** 1992. Safety and immunogenicity of single-dose live oral cholera vaccine CVD 103-HgR in 5 to 9-year-old Indonesian children. Lancet **340:**689–694.
35. **Tacket, C. O., G. Losonsky, J. P. Nataro, S. J. Cryz, R. Edelman, J. B. Kaper, and M. M. Levine.** 1992. Onset and duration of protective immunity in challenged volunteers after vaccination with live oral cholera vaccine CVD 103-HgR. J. Infect. Dis. **166:**837–841.
36. **Taylor, D. N., J. Rizzo, R. Meza, J. Perez, and D. Watts.** 1996. Cholera among Americans living in Peru. Clin. Infect. Dis. **22:**1108–1109.
37. **Weber, J. T., W. C. Levine, D. P. Hopkins, and R. V. Tauxe.** 1994. Cholera in the United States, 1965–1991. Risks at home and abroad. Arch. Int. Med. **154:**551–6.
38. **Wiedermann, G., H. Kollaritsch, E. Jeschko, M. Kundi, C. Herzog, and B. Wegmuller.** 1998. Adverse events after oral vaccination against cholera with CVD103-HgR. Wien. Klin. Wochenschr. **110:**376–378.
39. **Young, C. R., M. M. Levine, J. P. Craig, and R. Robins-Browne.** 1980. Microtiter enzyme-linked immunosorbent assay for immunoglobulin G cholera antitoxin in humans: method and correlation with rabbit skin vascular permeability factor technique. Infect. Immun. **27:**492–496.

Editor: D. L. Burns